GENE TRANSFER AND ELIMINATION IN BACTERIAL CROSSES WITH STRAIN K-12 OF *ESCHERICHIA COLI*

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Received for publication August 28, 1959

The possibility that in bacterial crosses only a fraction of the genetic material of the donor cell is physically transferred to the acceptor cell was suggested by Watson and Hayes (1953) and Hayes (1953). Later experiments of Jacob and Wollman, involving artificially interrupted mating (see review in Wollman *et al.*, 1956) with an Hfr (high frequency recombination) strain isolated by Hayes, supported this notion. Wollman and Jacob actually proposed that each Hfr strain is characterized by a specific point of rupture of the bacterial chromosome: in a cross only the genetic segment on one side of the rupture point would be physically transferred to the acceptor cell with high frequency. The general validity of this interpretation was contested, and alternatives proposed (see a discussion in Lederberg and Lederberg, 1956).

The experiments to be presented here were meant to test the idea of a specifically located region of rupture of the genetic chromosome of the Hayes Hfr strain. While this study was in progress, similar experiments were performed by Jacob and Wollman (1958) which compelled them to abandon the idea of a specific rupture region. Our results are nevertheless presented here as confirming Jacob and Wollman's latest results, as well as furnishing additional information on the transfer of several markers.

MATERIALS AND METHODS

Phages. Lambda and *lambda*-2 (Lederberg and Lederberg, 1953), high frequency transducing *lambda* (Arber, 1958), T1, T4, T5, and T6 (described in Adams, 1959) were used.

Bacteria. Escherichia coli strain K-12, and derivatives as listed in table 1 were used. Strain 4 was obtained in a rather devious way, involving several crosses between various derivatives of K-12: its markers L^-, T1,5^r, and Lac^- originate from an $F^-T^-L^-$, multiply marked strain developed originally by Lederberg; markers Gal_b^-, H^-, and C^- originate from the F^+ strain 112-12 of Wollman (1952); the three markers $T6^r$, T1,try^-, and Lp,Mal^- were introduced by selection of spontaneous mutants; Lp^+ was introduced by lysogenization; the F^+ factor was eliminated by growing the cells overnight in the presence of 200 μg/ml of acridine orange (method of Hirota, 1957).

Media. Media (described in detail by Bertani, 1957) were: Davis minimal medium, supplemented with 0.1 per cent L-asparagine, 0.5 per cent glucose, 5 μg/ml thiamine, amino acids in the amounts indicated by Lederberg (1950), and streptomycin to 200 μg/ml where indicated. LB agar (a rich nutrient agar); LB broth (LB agar without agar or calcium); nutrient agar; EMB agar supplemented with appropriate sugars.

For crosses, parent strains were incubated overnight in LB broth at 37 C with aeration, diluted 1:200 into LB broth, aerated 2½ hr at 37 C, counted in a Petroff-Hauser counting chamber, concentrated by centrifuging 20 min 3000 rpm, mixed to give approximately 1×10^8 males (Hfr) per ml and 5×10^8 females (F^-) per ml, incubated 2 hr at 37 C with gentle shaking on a rotary shaker, diluted, and plated on appropriate selective media to select for recombinants and also to assay for total male and female cells per ml. Controls of each parent alone were treated in the same manner.

To test for unselected markers, recombinant colonies were picked into sterile Aloe Willet aluminum caps (which had been filled with minimal media supplemented in the same way as the

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TABLE 1

Abbreviations used throughout paper:

$^+$	= wild type
^{s}	= sensitivity to phage or drug
C^-	= requires cystine for growth
Gal_b^-	= unable to ferment galactose
H^-	= requires histidine for growth
L^-	= requires leucine for growth
Lp^-	= nonlysogenic for phage *lambda*
Lac^-	= unable to ferment lactose
Lp,Mal^-	= unable to ferment maltose, and resistant to the virulent phage *lambda*-2 (simultaneous mutation)
M^-	= requires methionine for growth
S^r	= resistant to streptomycin
T^-	= requires threonine for growth
$T1^r$	= resistant to phage T1
$T4^r$	= resistant to phage T4
T1,5^r	= resistant to phages T1 and T5 (simultaneous mutation)
$T6^r$	= resistant to phage T6
T1,try^-	= resistant to phage T1, requires tryptophan for growth (simultaneous mutation)

Derivatives of Escherichia coli strain K-12 used:

1. Hfr Hayes Lp^- (Wollman *et al.*, 1956).
2. Hfr Hayes Lp^- S^r M^- (Hayes, 1953).
3. F^- L^- T1,5^r Lac^- $T6^r$ Gal_b^- T1,try^- H^- C^- Lp, Mal^- (see material and methods for derivation).
4. F^- T1,5^r Lac^- $T6^r$ Gal_b^- T1,try^- H^- C^- Lp, Mal^- (a recombinant obtained in a cross between strain 1 and strain 3).
5. F^- T1,5^r Lac^- $T6^r$ Gal_b^- T1,try^- H^- C^- S^r M^- (obtained as a recombinant in a cross between strain 1 and strain 4).

agar plate from which the recombinants were picked) and incubated overnight at 37 C. The caps were arranged in a pattern in petri dishes, and held in place with cotton. Two-inch long nails, fixed in the same pattern as the caps to a piece of wood, were used to replicate the recombinants to test agar media.

RESULTS AND DISCUSSION

Observations concerning the recombination mechanism in bacterial crosses. The following markers were utilized in the crosses to be described: L T1,5 Lac T6 Gal_b T1,try H C S M Hfr. The sequence in which they have been listed corresponds to the most likely sequence on the genetic chromosome. The left side of the genetic chromosome will refer to the L end, the right side to the Hfr end. For the markers L T1,5 Lac T6 Gal_b Lp H S M the sequence has been established by various workers (see discussion in Hartman, 1957, and also Jacob and Wollman, 1958). The position of Hfr is not certain. The position of the other markers will be discussed later.

In general agreement with the theory of progressive, oriented transfer of Hfr genes, a cross in which S^r was the F^- selective marker (table 2) showed that: (a) the frequency of transfer of any Hfr marker (table 2, columns 1 and 2) gradually decreases the further to the right the marker is located on the genetic chromosome; (b) in each class of recombinants obtained by selecting for a given Hfr marker, the unselected Hfr markers to the left of the selected Hfr marker appear in higher frequencies than the unselected Hfr markers located to the right of the selected Hfr marker. Exceptions to these two rules are discussed below.

The Hfr marker T1,try^+, which is located to the right of Gal_b^+, showed a frequency of transfer approximately equal to, if not greater than, that of Gal_b^+: the two markers, however, are not closely linked, since either marker appears in about half of the recombinants obtained by selecting for the other marker.[3] The transfer frequency of about 20 per cent observed for Gal_b^+ and T1,try^+ corresponds to the highest transfer frequency observed in similar experiments by Jacob and Wollman for markers located at the extreme left of the genetic chromosome. It is therefore probable that in these experiments the large majority of the transferred segments of the Hfr genetic chromosome include the locus T1,try and all loci to the left of it. If this is true, our experimental conditions correspond more precisely to the improved technique of Jacob and Wollman (1958, p. 506) than to their older technique in which a sharp gradient was noticeable in transfer of markers between L and Gal_b.

The improved technique of Jacob and Wollman consists essentially of (a) reducing agitation of the bacteria during the period of the cross; (b) removing and diluting very gently samples to be

[3] Gal_b^+ S^r recombinants were detected by plating approximately 400 cells from a cross mixture on EMB agar containing galactose and streptomycin, and noting dark colonies; this procedure permitted the detection of more Gal_b^+ S^r recombinants than did plating a larger number of cells from a cross on enriched minimal media containing galactose as the sole carbon source.

TABLE 2

Effect of selection for F⁻ marker Sʳ

Cross: Strain 1 Hfr + + + + Lp⁻ + + + + +
Strain 5 F⁻ T1, 5ʳ Lac⁻ T6ʳ Gal$_b$⁻ + T1, try⁻ H⁻ C⁻ Sʳ M⁻

Hfr marker selected	Recombinant colonies obtained (% input Hfr)	Frequency of unselected Hfr markers among recombinants (% of recombinant colonies showing the marker)										Total colonies scored
		T1, 5⁺	Lac⁺	T6⁺	Gal$_b$⁺	Lp⁻	T1, try⁺	H⁺	C⁺	M⁺	Hfr	
Gal$_b$⁺*	17	52	56	56		91	47	1.6	0	0	NS	123
T1, try⁺	21	30	33	33	52	45		0	0	0	0	105
H⁺	2.8	NS	35	NS	32	NS	47		17	0	NS	105
C⁺	0.53	NS	32	NS	42	NS	39	57		1.9	NS	52
M⁺*	0.044	3.8	0.96	0	0	0	1.9	0	2.9		47	104
None†		0	1.6	1.6	1.6	1.6	0	0	0	0	NS	63

* Recombinant colonies reisolated before analysis for unselected markers.

† At the end of the period of the cross, the cross mixture was plated on minimal medium containing streptomycin and all supplements required by the F⁻ for growth, and also on nutrient agar plus streptomycin. At least 60 per cent of the F⁻ cells which gave colonies on nutrient agar plus streptomycin gave colonies on the supplemented minimal agar, showing that many, but not all, viable F⁻ cells form colonies on appropriately supplemented minimal agar.

NS = not scored.

plated; and (c) prolonging the period of the cross to 80, 100, or 120 min.

Recombinants obtained by selecting for the Hfr marker M⁺ (which, in the Hfr Hayes strain, has the lowest frequency of transfer of any Hfr marker studied) do not show any appreciable percentage of the unselected Hfr markers located to the left of M (table 2). A fluctuation test (Jacob and Wollman, 1957) for the transfer of M⁺ by several sister Hfr cultures, grown up from small inocula, using the same strains used in the cross of table 2, gave no evidence of high variability in the number of M⁺ recombinants recovered. From this result, one might infer that M⁺ was not being transferred by different mutant Hfr clones newly arising in the original Hfr Hayes strain. A negative fluctuation test, however, might be obtained also if unstable mutant Hfr cells arose (a possibility suggested by Lederberg, 1959). Stimulation of M⁺ transfer by ultraviolet irradiation (Wollman *et al.*, 1956) would suggest that transfer of M⁺ may be by mutant Hfr strains.

Our results could also be explained by postulating a preferential rupture point or region between M and C, that is, much further to the right than the rupture point postulated by Wollman *et al.* (1956). M⁺ would then be on a segment of genetic material transferred separately from C and markers to the left of C in crosses.

Among recombinants obtained by selecting for Hfr markers located to the right of Lp, often unselected Hfr markers located to the left of the selected Hfr marker were present in less than 50 per cent of the recombinants (table 2). Since oriented transfer would insure the physical transfer of all such unselected Hfr markers, even an infinite amount of genetic crossing over should not lower their frequency of appearance among recombinants to less than 50 per cent. A figure lower than 50 per cent could be explained by irregular pairing between Hfr and F⁻ genetic material followed by elimination of Hfr genes from the zygote (Lederberg, 1949, 1957, 1959; Cavalli-Sforza and Jinks, 1956; Anderson, 1958).

When M⁺, rather than Sʳ, is used as the F⁻ selective marker (table 3): (a) there is less consistent decrease in frequency of transfer of Hfr markers as one goes from left to right on the genetic chromosome; (b) a higher percentage of recombinants show unselected Hfr markers to the right of the selected Hfr marker, and a lower percentage of recombinants show unselected Hfr markers to the left of the selected Hfr marker. Both of these differences tend to obscure any oriented transfer which may occur.

When Sʳ was the F⁻ selective marker, the mere presence (even without genetic incorporation) of the Hfr marker Sˢ in a zygote or in its early progeny may possibly have led to cell death, since Sˢ is dominant to Sʳ (Lederberg, 1951).

TABLE 3

Effect of selection for F⁻ marker M⁺

Cross A:	Strain 2 Hfr	+	+	+	+	+	+	+	S^r	M^-	+
	Strain 4 F^-	T1, 5^r	Lac^-	$T6^r$	Gal_b^-	T1, try^-	H^-	C^-	+	+	Lp, Mal^-
Cross B:	Strain 2 Hfr	+	+	+	+	+	+	+	+	M^-	+
	Strain 3 F^-	L^-	T1, 5^r	Lac^-	$T6^r$	Gal_b^-	T1, try^-	H^-	C^-	+	Lp, Mal^-

Hfr marker selected	Recombinant colonies obtained (% input Hfr)	Frequency of unselected Hfr markers among recombinants (% of recombinant colonies showing the marker)									Total colonies scored
		T1, 5^+	Lac^+	$T6^+$	Gal_b^+	T1, try^+	H^+	C^+	S^r	Lp, Mal^+	
Cross A											
T1, try^+	8	14	24	16	16		29	13	6	0	124
H^+	1.4	17	12	8	22	54		12	6	0	105
C^+	2.4	22	35	12	71	90	77		21	0	78
None*	1	7	1	1	1	4	0	0	0	0	105
Cross B											
L^+	18	53	22	10	5	9	3	0.8	0.8	NS	120

* At the end of the period of the cross, the cross mixture was plated on minimal medium containing all supplements required by the F^- for growth, and on nutrient agar. At least 60 per cent of the F^- cells which gave colonies on nutrient agar gave colonies on the supplemented minimal agar.

NS = not scored.

TABLE 4

Classification of $Gal_b^+S^r$ recombinants obtained in the cross of table 2

	Class of Gal_b^+ S^r Recombinants					
	$T5^s$	Lac^+	$T6^s$	Lp^-	T1, try^+	All Gal_b^+ S^r (recombinants)
$T5^s$ (from Hfr)		56	54	61	30	64
		— = 4.3	— = 3.6	— = 1.2	— = 1.1	— = 1.1
$T5^r$ (from F^-)		13	15	52	28	59
Lac^+ (from Hfr)	56		64	66	33	69
	— = 7		— = 12.8	— = 1.4	— = 1.3	— = 1.3
Lac^- (from F^-)	8		5	47	25	54
$T6^s$ (from Hfr)	55	64		66	34	69
	— = 6.1	— = 12.8		— = 1.4	— = 1.4	— = 1.3
$T6^r$ (from F^-)	9	5		46	24	54
Lp^- (from Hfr)	51	66	66		56	113
	— = 17	— = 22	— = 22		— = 56	— = 11.3
Lp^+ (from F^-)	3	3	3		1	10
T1, try^+ (from Hfr)	30	33	34	48		56
	— = 0.9	— = 0.9	— = 1	— = 0.8		— = 0.9
T1, try^- (from F^-)	34	36	35	64		65

Such killing could explain, in part, the decrease in frequency of transfer of Hfr markers observed as one examines Hfr markers nearer and nearer to S on the genetic chromosome (table 2). This effect would not occur when M^+ is the F^- selective marker (table 3), since M^+ is dominant to M^- (Lederberg, 1949).

Observations on the order of markers T1, try, H, and C. The sequence T1, try, H, C as written is consistent with the criteria listed earlier for

oriented transfer with S^r the F^- selective marker (table 2).

Since Gal_b^+ and T1,try$^+$ did not show any orientation with regard to each other, a detailed analysis was made of recombinants obtained by selecting for the Hfr marker Gal_b^+ and the F^- marker S^r, to establish the order of Gal_b and T1,try with regard to each other (table 4). All those $Gal_b^+S^r$ recombinants which showed a given unselected Hfr marker (e. g., all $T5^sGal_b^+S^r$ recombinants) were grouped in one class (in this case the $T5^s$ class). A sixth class consisted of *all* $Gal_b^+S^r$ recombinants. Within each class, for each of the other unselected Hfr markers, the ratio, "number of recombinants showing the Hfr allele of the marker"/"number of recombinants showing the F^- allele of the marker," was computed. The influence of incorporation of one unselected marker, say $T5^s$, on the presence of another, say Lac$^+$, may thus be seen by comparing the ratio Lac$^+$/Lac$^-$ in the $T5^s$ class (7) to the ratio Lac$^+$/Lac$^-$ among all Gal_b^+ recombinants (1.1). The lack of genetic interaction between Hfr markers to the left of Gal_b and the Hfr marker T1,try$^+$ strongly supports the order Gal_bT1,try.

Since Lp is at the right of Gal_b (Hartman, 1957), and 91 per cent of the $Gal_b^+S^r$ recombinants showed the unselected Hfr marker Lp$^-$, whereas only 47 per cent showed the unselected Hfr marker T1,try$^+$ (table 2), the order Gal_b Lp T1,try is indicated. The T1,try$^-$ marker is in the same order on the genetic chromosome as is the Try$^-$ mutation used by Jacob and Wollman (1958), as one would expect from the findings of Skaar and Davidson (1956) and Yanofsky and Lennox (1959). An attempt to transduce the T1,try locus, by means of high frequency transducing *lambda* failed.

Among recombinants selected for the Hfr marker C$^+$ with M$^+$ as the F^- selected marker (table 3), there is a much higher percentage of unselected markers H$^+$, T1,try$^+$, and Gal_b^+ (which are to the left of C) than there is of the unselected Hfr marker S^r which is to the right of H. Therefore it is indicated that S is to the right of C.

In crosses using S^r as the F^- selective marker, the marker Hfr did not appear among 105 recombinants selected for the Hfr marker T1,try$^+$, but did appear in 47 per cent of the recombinants selected for the Hfr marker M$^+$, indicating linkage between the markers M and Hfr, and lack of close linkage between Hfr and the other markers which were unlinked to M$^+$.

ACKNOWLEDGMENTS

The author wishes to thank Drs. G. and L. E. Bertani for their advice, and for aid in the preparation of this manuscript.

SUMMARY

Results of bacterial crosses utilizing the Hfr (high frequency of recombination) strain of Hayes are presented, which are consistent with the idea of oriented gene transfer (Jacob and Wollman, 1958), but also indicate the occurrence of postzygotic elimination (Lederberg, 1959). When M$^+$, rather than S^r, is used as the F^- selective marker, and the cross mixture incubated for 2 hr, any oriented transfer which might occur is not apparent.

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